(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 909 564 B1

(12) EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention of the grant of the patent:
21.06.2006 Bulletin 2006/25

(21) Application number: **97919693.8**

(22) Date of filing: **25.04.1997**

(51) Int Cl.:
A61K 38/18 *(2006.01)* ***A61K 47/16*** *(2006.01)*
A61K 9/08 *(2006.01)* ***C07K 14/505*** *(2006.01)*

(86) International application number:
PCT/JP1997/001449

(87) International publication number:
WO 1997/040850 (06.11.1997 Gazette 1997/47)

(54) ERYTHROPOIETIN SOLUTION PREPARATION STABILIZED WITH AMINO ACIDS

MIT AMINOSÄUREN STABILISIERTE ERYTHROPOIETINLÖSUNG

SOLUTION D'ERYTHROPOIETINE STABILISEE PAR DES ACIDES AMINES

(84) Designated Contracting States:
AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

(30) Priority: **26.04.1996 JP 13122696**
30.10.1996 JP 30395696

(43) Date of publication of application:
21.04.1999 Bulletin 1999/16

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Printed by Jouve, 75001 PARIS (FR)

Description

[0001] This invention relates to an erythropoietin solution preparation.

[0002] Erythropoietin (hereinafter referred to as EPO) is an acidic glycoprotein hormone which promotes the differentiation and proliferation of erythroid progenitor cells. This hormone is secreted chiefly by the kidney. Erythrocytes are present abundantly in the blood for certain periods, and are then destroyed by the spleen, etc. (their mean life in humans is about 120 days). However, red blood cells are constantly supplied from the bone marrow, so that the peripheral total erythrocyte count is kept constant in a normal state. EPO plays a central role in maintaining such homeostasis of erythrocytes in the living organism.

[0003] High purity human urinary EPO was obtained by purification from a large volume of urine from patients with aplastic anemia. This enabled cloning of human EPO gene. Nowadays, it has become possible to produce a large amount of recombinant human EPO in animal cells by genetic engineering technology. The applicant of this invention has succeeded in producing a preparation (lyophilized preparation) of the purified EPO, and supplies it to the market in the form of renal anemia alleviating agents and so on.

[0004] Drug design for supplying the market with stable EPO preparations requires that chemical changes (hydrolysis, disulfide exchange reaction, etc.) or.physical changes (denaturation, agglutination, adsorption, etc.) observed with EPO be suppressed. Products now on the market contain human serum albumin or purified gelatin which is generally used as a stabilizer. These substances have been added in these products to suppress chemical or physical changes. Since human serum albumin is a blood product relying on donated blood for its supply, the necessity for its addition has been questioned. Regarding the addition of a protein other than the albumin or gelatin as a stabilizer, it is difficult to avoid the risk of viral contamination completely.

[0005] Peptide drugs are often lyophilized for stabilization. However, lyophilization increases manufacturing costs, and involves an increased risk due to mechanical troubles.

[0006] For the foregoing reasons, demand is growing for an EPO preparation as an alternative to a lyophilized preparation, the EPO preparation being free from inclusion of a protein as a stabilizer, and stable during long-term storage.

[0007] EP-A-0178665 relates to an erythropoietin preparation containing one or more stabilizers selected from the group consisting of polyethylene glycol, protein, sugar, amino acid, inorganic salt, organic salt and a sulfur-containing reducing agent, wherein the amino acid stabilizer includes glycine, alanine and lysine.

[0008] EP-A-0430200 relates to a method for producing a pharmaceutical composition for subcutaneous or intramuscular administration comprising a polypeptide and at least one amino acid or a salt, derivate or homologous thereof.

[0009] EP-A-0306824 relates to a pharmaceutically acceptable human protein preparation having a long shelf life comprising a human protein, a physiologically acceptable buffer and optionally chelating agents, isotonicity adjusting agents, calcium chloride and other ingredients generally used for injection purposes, wherein said preparation contains 5 to 50 g/l urea, 1 to 50 g/l amino acid as well as 0,05 to 5 g/l of a nonionic wetting agent.

[0010] WO-A-9303744 relates to a process for producing preserved medicaments containing human proteins for the purposes of infusion or injection. Said medicament may contain erythropoietin as well as specific amino acids as stabilizers.

[0011] The inventors, have conducted extensive studies. As a result, they have found that EPO can be converted into a stable EPO solution preparation free from human serum albumin and purified gelatin by adding a certain amino acid as a stabilizer. This finding has led them to complete the present invention.

[0012] That is, the present invention provides an erythropoietin solution preparation containing an amino acid selected from the group consisting or histidine, tryptophan and serine as a stabilizing agent or stabilizer, and also relates to the use of one or more amino acids selected from the group consisting of tryptophan, serine, arginine and histidine as a stabilizer for an erythropoietin solution preparation, wherein said erythropoietin solution preparation does not contain urea.

[0013] "To stabilize; stabilizing" in this specification refers to storing, or the erythropoietin solution preparation, for example, for more than 2 years at 10°C, or for more than 6 months at 25°C, or for more than 2 weeks at 40°C while keeping the residual rate of erythropoietin at 90% or higher, preferably 95% or higher, more preferably 98% or higher.

Fig. 1 is a graph showing the relation between the concentration of L-arginine hydrochloride and the residual rate of erythropoietin;

Fig. 2 is a graph showing the relation between the concentration of L-lysine hydrochloride and the residual rate of erythropoietin;

Fig. 3 is a graph showing the relation between the concentration of L-histidine hydrochloride and the residual rate of erythropoietin; and

Fig. 4 shows an SDS-polyacrylamide gel electrophoresis pattern illustrating the degradation product suppressing effect of preparations to which various amino acids have been added (an electrophoretogram), in which lane 1: molecular weight marker, lane 2: amino acid-free preparation, lane 3: L-leucine-containing preparation, lane 4: 1-phenylalanine-containing preparation, lane 5: L-tryptophan- containing preparation, lane 6: L-serine-containing prep-

aration, lane 7: L-cysteine-containing preparation, lane 8: monosodium L-glutamate monohydrate-containing preparation, lane 9: L-arginine hydrochloride-containing preparation, and lane 10: L-histidine hydrochloride-containing preparation.

[0014] EPO for use in the solution preparation of the present invention has substantially the same biological activity as that of mammalian, especially, human EPO, and includes naturally occurring EPO and EPO obtained by genetic recombination. EPO from genetic recombination includes EPO having the same amino acid sequence as that of naturally occurring EPO, or EPO with this amino acid sequence from which one or more of the amino acids have been deleted, or in which one or more of the amino acids have been substituted, or to which one or more amino acids have been added, and which, however, retains the above-mentioned biological activity. The EPO in the present invention may be produced by any methods, for example, a method comprising extraction from human urine, followed by separation and purification, in various manners; and a method involving production in E. coli, yeast, or Chinese hamster ovary cells, followed by extraction, separation and purification in various manners.

[0015] The amino acid added as a stabilizer in the present invention includes free amino acids, and their salts such as sodium salts, potassium salts and hydrochlorides. The solution preparation of the present invention may have one or more of these amino acids added in combination. The amino acids are D-, L- and DL-forms of tryptophan, serine, arginine and histidine and their salts. More preferable are L-tryptophan, L-arginine and L-histidine and their salts. Particularly preferable are L-histidine and its salts.

[0016] The solution preparation of the present invention, preferably, is substantially free from protein as a stabilizer.

[0017] For the amount of the amino acid added to the solution preparation of the present invention, a preferred range can be set by a testing method (to be described later on) depending on the type of the amino acid used. Generally, the amount of the amino acid added is 0.001 to 50 mg/ml, but preferably 0.1 to 40 mg/ml, more preferably 1 to 10 mg/ml for arginine, preferably 0.5 to 10 mg/ml, more preferably 1.0 to 4.0 mg/ml, and most preferably 1.0 to 2.0 mg/ml for histidine. As will be described later on, the highest residual rate of EPO was obtained when L-arginine hydrochloride and L-lysine hydrochloride were each added in an amount of about 1 to 5 mg/ml as free amino acid, or when L-histidine hydrochloride was added in an amount, as free amino acid, of 1 to 10 mg/ml in an accelerated testing performed for 2 weeks at 40°C, or 0.5 to 5 mg/ml in a 25°C-6 month accelerated testing.

[0018] The amount of EPO contained in the solution preparation of the present invention can be determined according to the type of disease to be treated, the severity of the disease, the age of the patient, and so forth. Generally, its amount is 100 to 500,000 IU/ml, preferably 200 to 100,000 IU/ml, more preferably 750 to 72,000 IU/ml. The solution preparation of the present invention is administered usually by a parenteral route, for example, by injection (subcutaneous or intravenous), or percutaneously, transmucosally or transnasally, but oral administration is also possible.

[0019] The solution preparation of the present invention may contain, in addition to EPO and the amino acid, ingredients usually added to a preparation in the form of a solution, such as polyethylene glycol; sugars, e.g., dextran, mannitol, sorbitol, inositol, glucose, fructose, lactose, xylose, mannose, maltose, sucrose, and raffinose; inorganic salts, e.g., sodium chloride, potassium chloride, calcium chloride, sodium phosphate, potassium phosphate, and sodium hydrogen carbonate; organic salts, e.g., sodium citrate, potassium citrate and sodium acetate; and, if desired, sulfur-containing reducing agents, e.g., glutathione, thioctic acid, sodium thioglycolate, thioglycerol, α-monothioglycerol, and sodium thiosulfate. The preferred salt is sodium chloride. It is also preferred to add an adsorption preventing agent, such as a polyoxyethylene sorbitan alkyl ester, to the solution preparation of the present invention. Particularly preferable polyoxyethylene sorbitan alkyl esters are polysorbate 20, 21, 40, 60, 65, 80, 81 and 85, and most preferably, polysorbate 20 and/or 80. The preferred amount of polysorbate 20 and/or 80 added is 0.01 to 1 mg/ml, more preferably 0.05 to 0.1 mg/ml.

[0020] The solution preparation of the present invention can be prepared by dissolving the above-mentioned components in an aqueous buffer publicly known in the field of solution preparations, such as phosphate and/or citrate buffer. The preferred phosphate buffer is a sodium monohydrogen phosphate-sodium dihydrogen phosphate buffer, while the preferred citrate buffer is a sodium citrate buffer. The pH of the solution preparation of the present invention is 5.0 to 8.0, preferably, 6.0 to 7.0.

[0021] Japanese Unexamined Patent Publication No. 64-71818 discloses a human protein preparation characterized by containing urea, an amino acid, and a nonionic wetting agent. However, the solution preparation of the present invention preferably does not contain urea, because it is not clear whether urea contributes to the long-term stabilization of a glycoprotein such as erythropoietin. A reaction between urea degradation products and protein is also known to take place (Protein Chemistry 3, Kyoritsu Shuppan, Chapter 12), which may adversely affect the preparation. Furthermore, the fewer ingredients added to the preparation, the better the results that can be expected.

[0022] The solution preparation of the present invention is usually contained in a sealed, sterilized plastic or glass container. The solution preparation can be supplied as a prescribed dose in an ampoule, vial or disposable syringe, or in a multiple dose form such as a bag or bottle for injection.

[0023] EPO solution preparations containing various amino acids were prepared, and subjected to an accelerated testing conducted for 2 weeks at 40°C. The EPO content in each of the preparations after the test was measured by

RP-HPLC (reversed phase high performance liquid chromatography) to investigate the effect of amino acid addition on this content. As a result, the residual rate of EPO was found to be higher in the solution preparations containing L-leucine, L-tryptophan, monosodium L-glutamate monohydrate, L-arginine hydrochloride, L-histidine hydrochloride, and L-lysine hydrochloride than in the solution preparations containing no amino acids. The results of SDS-polyacrylamide gel electrophoresis demonstrated L-arginine hydrochloride and L-histidine hydrochloride to be effective in suppressing the formation of EPO degradation products to be observed in the preparation after the accelerated testing.

[0024] Of the amino acids thus shown to be effective when added, L-arginine hydrochloride, L-lysine hydrochloride and L-histidine hydrochloride were examined for the effect of their concentrations on the stabilization of the preparation. That is, EPO preparations to which L-arginine hydrochloride, L-lysine hydrochloride or L-histidine hydrochloride was added in various concentrations were made, and a 40°C-2 week accelerated testing was conducted on these preparations. Upon completion of the test, the residual rates of EPO in the preparations tended to peak at concentrations of about 1 to 5 mg/ml in the case of L-arginine hydrochloride and L-lysine hydrochloride. With L-histidine hydrochloride, maximum EPO residual rate was achieved at a concentration of 1 to 10 mg/ml. A 25°C-6 month accelerated testing was also performed in EPO preparations to which L-histidine hydrochloride was added in various concentrations. The EPO residual rate was maximal at the concentrations of 0.5 to 5 mg/ml. These findings showed L-arginine hydrochloride, L-lysine hydrochloride, and L-histidine hydrochloride to have the optimum concentration of addition.

[0025] The EPO solution preparation of the present invention is a safe preparation free from foreign proteins such as human serum albumin or purified gelatin, and without the risk of viral contamination. The amino acid added thereto is cheaper than these conventional stabilizers, and the cost incurred during the manufacturing process is also lower than that for a lyophilized product. Thus, the preparation of this invention is advantageous economically. Furthermore, the solution preparation of the present invention need not be dissolved in a buffer, but can be used as it is. This lessens labor in using it in comparison with a lyophilized preparation. Because of these various advantages, the industrial applicability of the present invention is great.

[0026] The present invention will now be described in further detail by reference to the following examples, but its scope is not restricted thereby.

Examples

Testing method

[0027] A 5 ml glass vial was charged with 1 ml of a dispensing solution containing the following components/ml and being adjusted to pH 6.0 with a 10 mM phosphate buffer (Wako Pure Chemical Industries, Ltd.):

EPO	1,500 IU
Nonionic surfactant (polysorbate 80, Nikko Chemical Co., Ltd.)	0.05 mg
Sodium chloride	8.5 mg
Amino acid (Sigma Chemical Company)	0 to 40 mg

The filled vial was stoppered, sealed, and used as a solution preparation. As an accelerated testing, the preparation was allowed to stand for 2 weeks in a thermostatic chamber at 40°C. Then, the preparation was evaluated by RP-HPLC analysis (WATERS) and SDS-polyacrylamide gel electrophoresis analysis.

Example 1: Effect of the addition of various amino acids on EPO residual rate

[0028] In accordance with the foregoing testing method, the solution preparations containing various amino acids tabulated below were produced, and subjected to the 40°C-2 week accelerated testing. Then, their EPO residual rates were determined by the RP-HPLC method. The results are shown in Table 1.

Table 1 EPO residual rates after accelerated testing of various amino acids-containing solution preparations

Amino acid	Amount added (mg/ml)	EPO residual rate after 40°C-2 week accelerated testing (% of initial content)
Not added	0	83.9
L-leucine	10	91.6
L-phenylalanine	10	57.8

Table continued

Amino acid	Amount added (mg/ml)	EPO residual rate after 40°C-2 week accelerated testing (% of initial content)
L-tryptophan	5	97.0
L-serine	10	85.2
L-cysteine	10	47.1
Monosodium L-glutamate monohydrate	10	93.9
L-arginine hydrochloride	10	93.6
L-histidine hydrochloride	10	99.7
L-lysine hydrochloride	. 10	95.8

[0029] As shown above, L-leucine, L-tryptophan, monosodium L-glutamate monohydrate, L-arginine hydrochloride, L-histidine hydrochloride, and L-lysine hydrochloride led to particularly marked EPO residual rates. Monosodium L-glutamate monohydrate, L-leucine, L-phenylalanine, L-cyskine and L-lysine hydrochloride are not comprised by the claimed subject-matter of the present application.

Example 2: Effect of the addition of an amino acid in various concentrations on EPO residual rate

[0030] In accordance with the foregoing testing method, EPO solution preparations containing L-arginine hydrochloride in various concentrations indicated below were produced, and subjected to the same 40°C-2 week accelerated testing. Then, their EPO residual rates were determined by the RP-HPLC method. The results are shown in Table 2.

Table 2 EPO residual rates after accelerated testing of L-arginine hydrochloride-containing preparations

Amino acid	Amount added (mg/ml) (mg/ml)	EPO residual rate after 40°C-2 week accelerated testing (% of initial content)
Not added	0	89.6
L-arginine hydrochloride	0.1	92.7
	1	96.7
	5	96.1
	10	93.6
	20	92.0
	40	91.6

[0031] The above results are depicted as a graph in Fig. 1.

[0032] As shown above, L-arginine hydrochloride led to maximum EPO residual rates in a concentration range of about 1 to 5 mg/ml.

[0033] Then, the same test was conducted using L-lysine hydrochloride. The amounts of L-lysine hydrochloride added and the EPO residual rates after the accelerated testing are shown in Table 3.

Table 3 EPO residual rates after accelerated testing of L-lysine hydrochloride-containing preparations

Amino acid	Amount added (mg/ml) (mg/ml)	EPO residual rate after 40°C-2 week accelerated testing (% of initial content)
Not added	0	88.7
L-lysine hydrochloride	0.5	93.1
	1	95.8
	5	96.3
	10	90.2

[0034] The above results are depicted as a graph in Fig. 2.

[0035] As shown above, L-lysine hydrochloride also led to maximum EPO residual rates in a concentration range of about 1 to 5 mg/ml.

[0036] Then, the same test was conducted using L-histidine hydrochloride. The amounts of L-histidine hydrochloride added and the EPO residual rates after the accelerated testing are shown in Table 4.

Table 4 EPO residual rates after accelerated testing of L-histidine hydrochloride-containing preparations

Amino acid	Amount added (mg/ml)	EPO residual rate after 40°C-2 week accelerated testing (% of initial content)
Not added	0	91.5
L-histidine hydrochloride	0.5	95.5
	1	97.3
	5	98.1
	10	99.7

[0037] The above results are depicted as a graph in Fig. 3.

[0038] As shown above, L-histidine hydrochloride led to maximum EPO residual rates in a concentration range of about 1 to 10 mg/ml.

[0039] In accordance with the aforementioned testing method, EPO solution preparations containing L-histidine hydrochloride in various concentrations indicated below were produced, and subjected to a 25°C-6 month accelerated testing. Then, their EPO residual rates were determined by the RP-HPLC method. The results are shown in Table 5.

Table 5 EPO residual rates after accelerated testing of L-histidine hydrochloride-containing preparations

Amino acid	Amount added (mg/ml)	EPO residual rate after 25°C-6 month accelerated testing (% of initial content)
Not added	0	93.2
L-histidine hydrochloride	0.5	99.3
	1	99.9
	5	97.9
	10	94.1

[0040] As shown above, L-histidine hydrochloride led to maximum EPO residual rates in a concentration range of 0.5 to 5 mg/ml, especially, at a concentration of 1 mg/ml.

Example 3: Effect of the addition of various amino acids on EPO degradation products

[0041] In accordance with the aforementioned testing method, EPO solution preparations containing various amino acids were produced, and subjected to a 40°C-2 week accelerated testing. Then, the formation of EPO degradation products was investigated by the SDS-polyacrylamide gel electrophoresis analysis method.

1) Preparation of sample

[0042] After the accelerated testing, a 1M TRIS-hydrochloride buffer (pH 6.8) containing SDS, glycerin, and Bromophenol Blue was added to each of the EPO solution preparations containing each of the various amino acids indicated in Table 1 of Example 1. The mixture was heated for 15 minutes at 60°C for use as a sample solution.

2) Electrophoresis

[0043] The sample solution (10 μl) was electrophoresed under the following operating conditions:

a) Equipment: Slab gel electrophoresis apparatus (Bio-Rad Laboratories)

b) Electrophoresis gel:

SDS-PAGEmini8-16 (concentration gradient gel in polyacrylamide concentrations of 8 to 16%, Tefco)

c) Electrophoresis temperature: 25°C
d) Electrophoresis conditions:

25 mA constant current/gel

3) Staining method (western blotting)

[0044] The electrophoresed gel was transferred to a polyvinylidene difluoride membrane. Then, anti-EPO rabbit antiserum, biotin-labeled anti-rabbit IgG goat antibody, and biotinylated horseradish peroxidase were used for color development with 3,3'-diaminobenzidine-hydrogen peroxide as a substrate.

4) Results

[0045] The results obtained are shown in Fig. 4. Compared with the amino acid-free preparation (lane 2), the monosodium L-glutamate monohydrate-containing preparation (lane 8), the L-arginine hydrochloride-containing preparation (lane 9), and the L-histidine hydrochloride-containing preparation (lane 10) showed the marked effect of suppressing the formation of EPO degradation products.

Claims

1. An erythropoietin solution preparation, which contains as a stabilizer one or more amino acids selected from the group consisting of histidine, tryptophan and serine, and their salts.

2. The solution preparation of claim l,wherein the stabilizer is one or more amino acids selected from the group consisting of L-histidine, L-tryptophan, and L-serine, and their salts.

3. The solution preparation of claim 1, wherein the stabilizer is one or more amino acids selected from the group consisting of histidine and tryptophan, and their salts.

4. The solution preparation of claim 1, wherein the stabilizer is histidine and/or its salts.

5. The solution preparation of any one of claims 1 to 4, wherein the concentration of the amino acid is 0.1 to 40 mg/ml.

6. The solution preparation of any one of claims 1 to 4, wherein the concentration of the histidine is 0.5 to 5 mg/ml.

7. The solution preparation of any one of claims 1 to 6, which does not include urea.

8. The solution preparation of any one of claims 1 to 7, which does not contain a protein as a stabilizer.

9. The solution preparation of any one of claims 1 to 8, which further contains a surfactant.

10. The solution preparation of claim 9, wherein the surfactant is a polyoxyethylene sorbitan alkyl ester.

11. The solution preparation of claim 9, wherein the surfactant is polysorbate 20 and/or 80.

12. The solution preparation of any one of claims 1 to 11, which further contains a salt.

13. The solution preparation of claim 12, wherein the salt is sodium chloride.

14. The solution preparation of any one of claims 1 to 13 which has been dissolved in a buffer.

15. The solution preparation of claim 14, wherein the buffer is a phosphate buffer and/or a citrate buffer.

16. A method for stabilizing an erythropoietin solution preparation, which comprises adding a stabilizer which contains one or more amino acids selected from the group consisting of tryptophan, histidine and serine, and their salts, to the erythropoietin solution preparation.

17. A method for stabilizing an erythropoietin solution preparation, which comprises adding a stabilizer which contains one or more amino acids selected from the group consisting of tryptophan, serine, arginine and histidine, and their salts, and which does not contain urea, to the erythropoietin solution preparation.

18. Use of one or more amino acids selected from the group consisting of tryptophan, serine, arginine and histidine, and their salts as a stabilizer for an erythropoietin solution preparation, wherein said erythropoietin solution preparation does not contain urea

Patentansprüche

1. Erythropoietinlösungszubereitung, welche eine oder mehrere Aminosäuren, ausgewählt aus Histidin, Tryptophan und Serin und deren Salzen, als Stabilisator enthält.

2. Lösungszubereitung gemäß Anspruch 1, wobei der Stabilisator eine oder mehrere Aminosäuren ist, ausgewählt aus L-Histidin, L-Tryptophan und L-Serin und deren Salzen.

3. Lösungszubereitung gemäß Anspruch 1, wobei der Stabilisator eine oder mehrere Aminosäuren ist, ausgewählt aus Histidin und Tryptophan und deren Salzen.

4. Lösungszubereitung gemäß Anspruch 1, wobei der Stabilisator Histidin und/oder dessen Salze ist.

5. Lösungszubereitung gemäß einem der Ansprüche 1 bis 4, wobei die Konzentration der Aminosäure 0,1 bis 40 mg/ml beträgt.

6. Lösungszubereitung gemäß einem der Ansprüche 1 bis 4, wobei die Konzentration des Histidins 0,5 bis 5 mg/ml beträgt.

7. Lösungszubereitung gemäß einem der Ansprüche 1 bis 6, welche keinen Harnstoff enthält.

8. Lösungszubereitung gemäß einem der Ansprüche 1 bis 7, welche kein Protein als Stabilisator enthält.

9. Lösungszubereitung gemäß einem der Ansprüche 1 bis 8, welche weiterhin ein grenzflächenaktives Mittel enthält.

10. Lösungszubereitung gemäß Anspruch 9, wobei das grenzflächenaktive Mittel Polyoxyethylensorbitanalkylester ist.

11. Lösungszubereitung gemäß Anspruch 9, wobei das grenzflächenaktive Mittel Polysorbat 20 und/oder 80 ist.

12. Lösungszubereitung gemäß einem der Ansprüche 1 bis 11, welches weiterhin ein Salz enthält.

13. Lösungszubereitung gemäß Anspruch 12, wobei das Salz Natriumchlorid ist.

14. Lösungszubereitung gemäß einem der Ansprüche 1 bis 13, welche in einem Puffer gelöst ist.

15. Lösungszubereitung gemäß Anspruch 14, wobei der Puffer ein Phosphatpuffer und/oder ein Citratpuffer ist.

16. Verfahren zum Stabilisieren einer Erythropoietinlösungszubereitung, welches Zugeben eines Stabilisators, der eine oder mehrere aus Tryptophan, Histidin und Serin und deren Salzen ausgewählte Aminosäuren enthält, zu der Erythropoietinlösungszubereitung umfasst.

17. Verfahren zum Stabilisieren einer Erythropoietinlösungszubereitung, welches Zugeben eines Stabilisators, der eine oder mehrere aus Tryptophan, Serin, Arginin und Histidin und deren Salzen ausgewählte Aminosäuren enthält und der keinen Harnstoff enthält, zu der Erythropoietinlösungszubereitung umfasst.

18. Verwendung einer oder mehrerer Aminosäuren, ausgewählt aus Tryptophan, Serin, Arginin und Histidin und deren Salzen, als Stabilisator für eine Erythropoietinlösungszubereitung, wobei die Erythropoietinlösungszubereitung keinen Harnstoff enthält.

Revendications

1. Préparation d'érythropoïétine en solution, qui contient, en tant que stabilisant, un ou plusieurs acides aminés sélectionnés dans le groupe constitué par l'histidine, le tryptophane et la sérine, ainsi que leurs sels.

2. Préparation en solution selon la revendication 1, dans laquelle le stabilisant est un ou plusieurs acides aminés sélectionnés dans le groupe constitué par la L-histidine, le L-tryptophane, et la L-sérine, ainsi que leurs sels.

3. Préparation en solution selon la revendication 1, dans laquelle le stabilisant est un ou plusieurs acides aminés sélectionnés dans le groupe constitué par l'histidine et le tryptophane, ainsi que leurs sels.

4. Préparation en solution selon la revendication 1, dans laquelle le stabilisant est l'histidine et/ou ses sels.

5. Préparation en solution selon l'une quelconque des revendications 1 à 4, dans laquelle la concentration de l'acide aminé est de 0,1 à 40 mg/ml.

6. Préparation en solution selon l'une quelconque des revendications 1 à 4, dans laquelle la concentration de l'histidine est de 0,5 à 5 mg/ml.

7. Préparation en solution selon l'une quelconque des revendications 1 à 6, qui ne contient pas d'urée.

8. Préparation en solution selon l'une quelconque des revendications 1 à 7, qui ne contient pas de protéine en tant que stabilisant.

9. Préparation en solution selon l'une quelconque des revendications 1 à 8, qui contient en outre un tensioactif.

10. Préparation en solution selon la revendication 9, dans laquelle le tensioactif est un ester alkylique de sorbitan polyoxyéthyléné.

11. Préparation en solution selon la revendication 9, dans laquelle le tensioactif est le polysorbate 20 et/ou 80.

12. Préparation en solution selon l'une quelconque des revendications 1 à 11, qui contient en outre un sel.

13. Préparation en solution selon la revendication 12, dans laquelle le sel est le chlorure de sodium.

14. Préparation en solution selon l'une quelconque des revendications 1 à 13, qui a été dissoute dans un tampon.

15. Préparation en solution selon la revendication 14, dans laquelle le tampon est un tampon phosphate et/ou un tampon citrate.

16. Procédé pour stabiliser une préparation d'érythropoïétine en solution, qui comprend l'addition d'un stabilisant qui contient un ou plusieurs acides aminés sélectionnés dans le groupe constitué par le tryptophane, l'histidine et la sérine, ainsi que leurs sels, à la préparation d'érythropoïétine en solution.

17. Procédé pour stabiliser une préparation d'érythropoïétine en solution, qui comprend l'addition d'un stabilisant qui contient un ou plusieurs acides aminés sélectionnés dans le groupe constitué par le tryptophane, la sérine, l'arginine et l'histidine, ainsi que leurs sels, et qui ne contient pas d'urée, à la préparation d'érythropoïétine en solution.

18. Utilisation d'un ou plusieurs acides aminés sélectionnés dans le groupe constitué par le tryptophane, la sérine, l'arginine et l'histidine, ainsi que leurs sels, en tant que stabilisant pour une préparation d'érythropoïétine en solution, dans laquelle ladite préparation d'érythropoïétine en solution ne contient pas d'urée.

Fig. 1

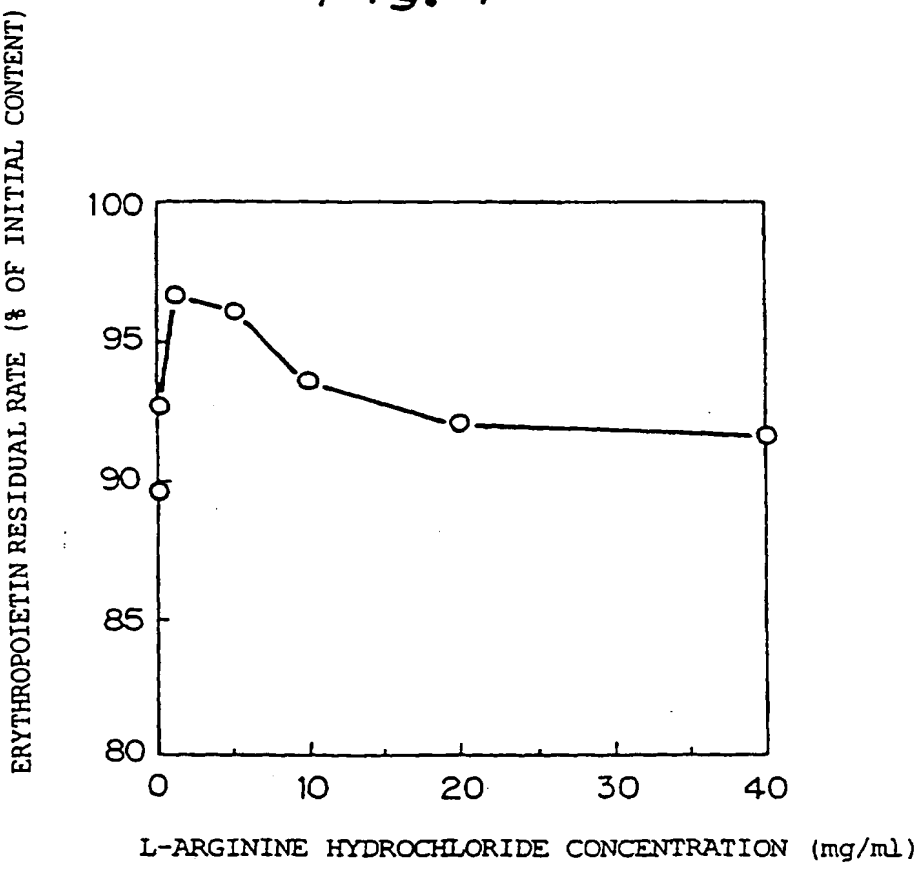
ERYTHROPOIETIN RESIDUAL RATE (% OF INITIAL CONTENT)
100
95
90
85
80
0
10
20
30
40
L-ARGININE HYDROCHLORIDE CONCENTRATION (mg/ml)

Fig. 2

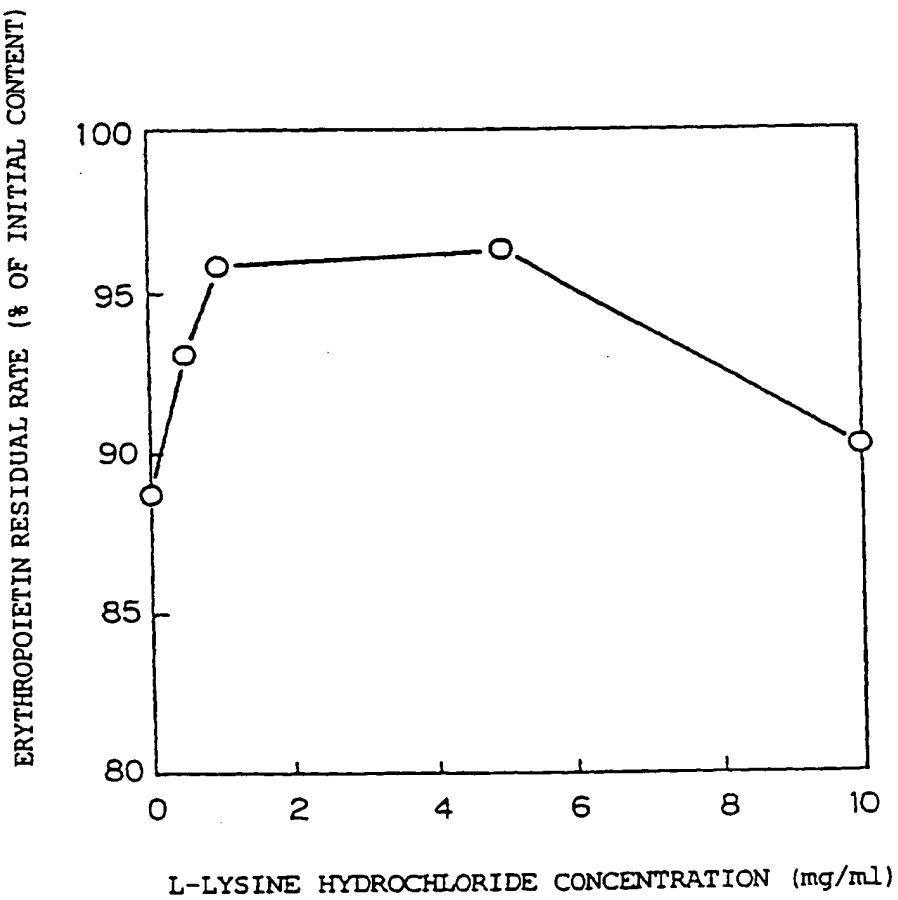
ERYTHROPOIETIN RESIDUAL RATE (% OF INITIAL CONTENT)
100
95
90
85
80
0
2
4
6
8
10
L-LYSINE HYDROCHLORIDE CONCENTRATION (mg/ml)

Fig. 3

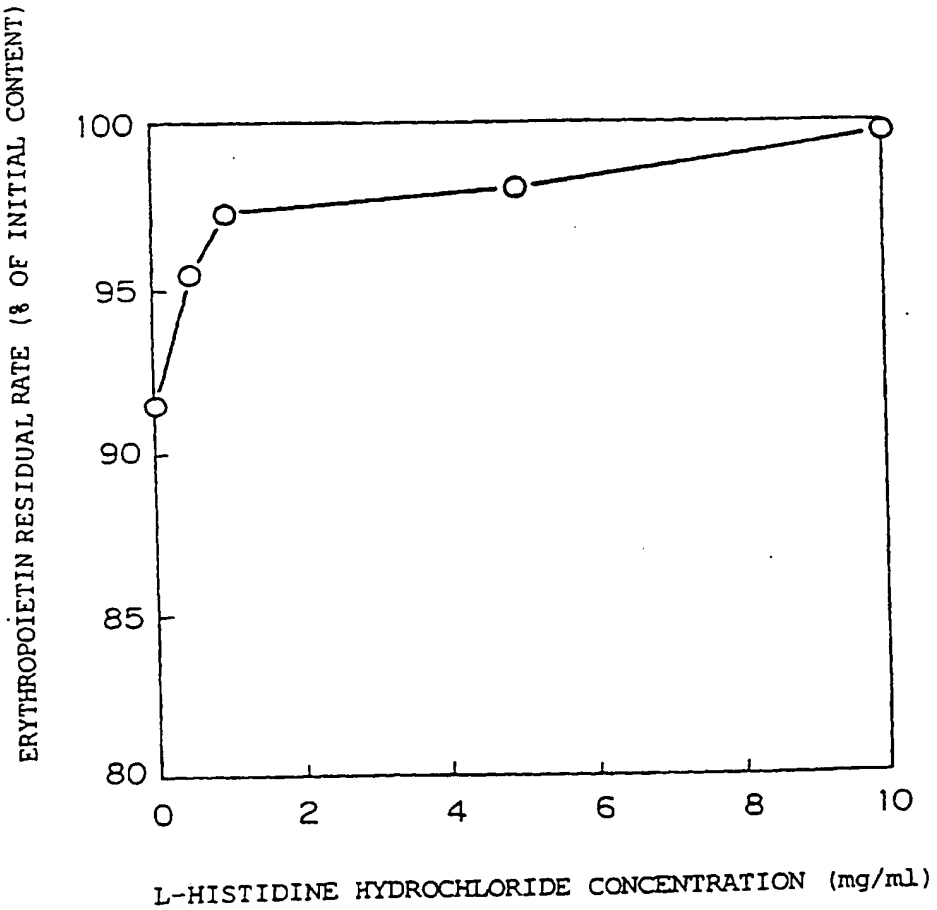
ERYTHROPOIETIN RESIDUAL RATE (% OF INITIAL CONTENT)
100
95
90
85
80
0
2
4
6
8
10
L-HISTIDINE HYDROCHLORIDE CONCENTRATION (mg/ml)

Fig. 4